

Marina Montosa Belluci  
 Gabriela Giro  
 Ricardo Andrés Landazuri  
 del Barrio  
 Rosa Maria Rodrigues Pereira  
 Elcio Marcantonio Jr  
 Silvana Regina Perez Orrico

## Effects of magnesium intake deficiency on bone metabolism and bone tissue around osseointegrated implants

### Authors' affiliations:

Marina Montosa Belluci, Gabriela Giro, Ricardo Andrés Landazuri del Barrio, Elcio Marcantonio Jr, Silvana Regina Perez Orrico, Department of Oral Diagnosis and Surgery, Araraquara Dental School – UNESP, University of Estadual Paulista, Araraquara, São Paulo, São Paulo, Brazil  
 Rosa Maria Rodrigues Pereira, Bone Metabolism Laboratory of Rheumatology Division, Faculdade de Medicina da Universidade de São Paulo, São Paulo, São Paulo, Brazil

### Corresponding author:

Silvana Regina Perez Orrico  
 Departamento de Diagnóstico e Cirurgia  
 Faculdade de Odontologia de Araraquara – UNESP,  
 Rua Humaitá 1680 14801-403  
 Araraquara  
 São Paulo  
 Brazil  
 Tel.: + 55 16 3301 6377  
 Fax: + 55 16 3301 6369  
 e-mail: s-orrico@foar.unesp.br

**Key words:** bone markers, dental implants, magnesium deficiency, rat

### Abstract

**Objectives:** This study evaluated the effect of magnesium dietary deficiency on bone metabolism and bone tissue around implants with established osseointegration.

**Materials and methods:** For this, 30 rats received an implant in the right tibial metaphysis. After 60 days for healing of the implants, the animals were divided into groups according to the diet received. Control group (CTL) received a standard diet with adequate magnesium content, while test group (Mg) received the same diet except for a 90% reduction of magnesium. The animals were sacrificed after 90 days for evaluation of calcium, magnesium, osteocalcin and parathyroid hormone (PTH) serum levels and the deoxypyridinoline (DPD) level in the urine. The effect of magnesium deficiency on skeletal bone tissue was evaluated by densitometry of the lumbar vertebrae, while the effect of bone tissue around titanium implants was evaluated by radiographic measurement of cortical bone thickness and bone density. The effect on biomechanical characteristics was verified by implant removal torque testing.

**Results:** Magnesium dietary deficiency resulted in a decrease of the magnesium serum level and an increase of PTH and DPD levels ( $P \leq 0.05$ ). The Mg group also presented a loss of systemic bone mass, decreased cortical bone thickness and lower values of removal torque of the implants ( $P \leq 0.01$ ).

**Conclusions:** The present study concluded that magnesium-deficient diet had a negative influence on bone metabolism as well as on the bone tissue around the implants.

Magnesium is one of the most abundant minerals in the body and is essential for several enzymes and cell functions, acting as an important modifier of the inflammatory and immune response (Maguire & Cowan 2002). It also plays a relevant role on bone tissue and mineral homeostasis and may directly affect the function of bone cells and the hydroxyapatite crystal growth (Creedon et al. 1999).

It is estimated that from 2.5 to 15% of the world population suffers from some form of hypomagnesemia (Sabbagh et al. 2008). Magnesium dietary deficiency may be common in industrialized countries, as reported in the United States (Marx & Neutra 1997; Ford & Mokdad 2003) and European countries (Schimatschek & Rempis 2001; Touvier et al. 2006). Although it is a mineral found in many foods, its absorption requires ideal conditions and may be easily inhibited by several factors. Even after being absorbed in the body, several substances contribute to an increased kidney excretion of magnesium

such as excessive alcohol intake, diuretics, coffee, tea, salt, phosphoric acid and sugar, all common in diets nowadays (Johnson 2001). Some manifestations may be related to a deficiency such as hypertension, vascular function alteration, insulin resistance and/or altered insulin secretion (Evangelopoulos et al. 2008).

Some epidemiological studies show a positive correlation between a magnesium-deficient diet and an increase in loss of bone mass and/or a decrease in bone density, which suggest that this mineral deficiency may be a risk factor for osteoporosis (New et al. 1997; Tucker et al. 1999; Wang et al. 1999). Animal studies with different levels of deficiency showed bone loss, characterized by a decrease of trabecular bone volume, followed by an increase in the release of pro-inflammatory cytokines and alteration in secretion and action of the parathyroid hormone (PTH), contributing to the decrease in bone formation (Rude et al. 2004, 2005, 2006). For a prolonged period (12 months), a magnesium

### Date:

Accepted 13 July 2010

### To cite this article:

Belluci MM, Giro G, del Barrio RAL, Pereira RMR, Marcantonio E Jr, Orrico SRP. Effects of magnesium intake deficiency on bone metabolism and bone tissue around osseointegrated implants.  
*Clin. Oral Impl. Res.* 22, 2011; 716–721.  
 doi: 10.1111/j.1600-0501.2010.02046.x

deficient-diet induced the bone mass loss of lumbar vertebrae and femurs in addition to biomechanical and histomorphometric changes of bone tissue in rats, similar to those in human osteoporosis (Stendig-Lindberg et al. 2004). For this reason, it was hypothesized that bone changes caused by magnesium deficiency could be a risk factor for the maintenance of the implants to the extent that they affect bone remodeling.

The purpose of this study was to evaluate the effect of magnesium deficiency on systemic bone metabolism and bone tissue around implants with established osseointegration.

## Materials and methods

### Animals

This study was approved by the Animal Experimentation Ethics Committee of the Araraquara Dental School – UNESP (Protocol #20/2006).

Thirty 60-day-old rats (*Rattus norvegicus albinus*, Holtzman) were used in this study. The animals were kept in individual stainless-steel cages in the animal facility with controlled temperature, humidity and light exposure.

Animals were divided into two groups ( $n = 15$ ) according to their diet. The control group (CTL) was fed on a diet with standard daily magnesium content (507 mg/kg of Mg) according to AIN-93 (American Institute of Nutrition) for maintenance of rodents (Reeves et al. 1993; Reeves 1997). The test group (Mg) was fed on a diet with 90% reduction in the magnesium content (50.7 mg/kg of Mg). Body weight was monitored weekly to assess the animal's growth.

### Study design (Fig. 1)

On day 0, all animals underwent surgery for implant placement in the left tibia. The animals received the standard diet during the 60 days required for the healing of the implants (Clokie & Warshawsky 1995). For the next 90 days, the control group continued to receive the standard diet, while the Mg group was given the standard diet with magnesium reduction. At 150 days, all animals were sacrificed by deep anesthesia.

### Surgical procedure

The animals were anesthetized with a combination of ketamine chloridrate (Ketamina Agener, Agener União Ltda, São Paulo, São Paulo, Brazil) at a concentration of 0.08 ml/100 g body weight and 2% xylazine chloridrate (Rompum, Bayer S.A., São Paulo, São Paulo, Brazil) at a concentration of 0.04 ml/100 g. Next, the animals were submitted to preoperative trichotomy in the inner region of the leg and aseptics with povidone iodine solution.

An incision was made in layers on the tibial metaphysis. The underlying bone was subjected to osteotomy under abundant irrigation, carried out with a start drill of 1.8 mm for the accommodation of the titanium implant (aluminum sand blasting and acid-etched surface), 4 mm long and 2.2 mm in thickness (Conexão Sistemas de Protéses Ltda, Aruja, São Paulo, Brazil). The tissue was sutured with silk thread 4-0 (Ethicon, Division of Johnson & Johnson Medical Limited, São Jose dos Campos, São Paulo, Brazil).

The animals received an intramuscular dose of penicillin associated with streptomycin (Pentabiotic Pequeno Porte, Fort Dodge<sup>®</sup>, Campinas, São Paulo, Brazil), 0.1 ml/kg of bodyweight and 5 mg/kg of dexamethasone intramuscular (Dexter, Agener<sup>®</sup>, Agener União Ltda, São Paulo, São Paulo, Brazil) immediately after the surgery.

### Biochemical evaluations

The animals were kept in metabolic cages for urine collection during 24 h before sacrifice for a later dosage of deoxypyridinoline (DPD) (DPD Metra<sup>®</sup>, Quidel Corporation, San Diego, CA, USA) level by ELISA method.

At sacrifice, a blood sample was taken by caudal artery puncture for the determination of magnesium (Magnésio, Labtest Diagnostica SA, Lagoa Santa, MG, Brazil) and calcium (Cálcio Arsenazo Liquiform, Labtest Diagnostics SA, Lagoa Santa, MG, Brazil) serum levels by the colorimetric method. Osteocalcin (OCN) (Rat Osteocalcin EIA Kit, Biomedical Technologies Inc., Stoughton, MA, USA) and PTH (Rat Total Intact PTH ELISA Kit, Scantibodies Laboratory Inc., Santee, CA, USA) serum levels were evaluated by the ELISA method.

### Bone densitometry

Bone densitometry was obtained by dual-energy X-ray absorptiometry (DXA) using a densitometer (Discovery-A SN: 80999 Hologic, Bedford, MA, USA) in the high-resolution mode and analyzed by the Small Animal software, supplied by the equipment manufacturer. For this, overall BMD measurements were taken as well as those of the lumbar vertebrae 2 (L2), 3 (L3) and 4 (L4).

DXA accuracy for the determination of BMD was evaluated by the coefficient of variation, expressed as a percentage of the average (Grier et al. 1996). For this, five consecutive measurements of each anatomical region of the same sample were made. The coefficient of variation obtained was 1.9%.

### Image acquisition

Radiographic images of the implants were obtained by a direct digital imaging system – CDR (Computed Dental Radiography for Microsoft Windows, Shick<sup>®</sup> Technologies Inc., Long Island, NY, USA). In order to standardize images, tibia with implants and a sensor were placed into a rigid positioning device where the long axis of the implant was perpendicular to the central X-ray beam and parallel to the sensor at a focus distance of 40 cm. The sensor was exposed to X-rays of 70 kVp and 10 mA for a 15 pulses/s exposure period. Images were stored in TIFF (Tagged Image File Format) without image compression and analyzed by an image analyzer software (Adobe<sup>®</sup> Photoshop<sup>®</sup> CS2 9.0, Adobe System Incorporated, San Jose, CA, USA).

### Radiographic bone density

A single blinded calibrated examiner evaluated tibia radiographies. Radiographic bone density was obtained by measuring the gray level (histogram) in an area of  $5 \times 5$  pixels at six different points, cortical (upper and lower) and medullar region, on both sides of the implant (Fig. 2).

Calculation of bone density was performed by first obtaining the average of gray level values in each region of interest and then the values of gray level of the implant. The value of the regions of interest was divided by the relative value of the implant to compensate small differences among radiographies.

### Cortical bone thickness

The thickness of the tibia cortical bone (upper and lower) was assessed by radiographic images by a blinded calibrated examiner. The measured region was standardized at 1 mm from the implant. An image analyzer software (UTHSCSA ImageTool, San Antonio, TX, USA) was used for linear measurement in millimeters of each cortical on both sides of the implant. Mean values were obtained for each cortical bone.

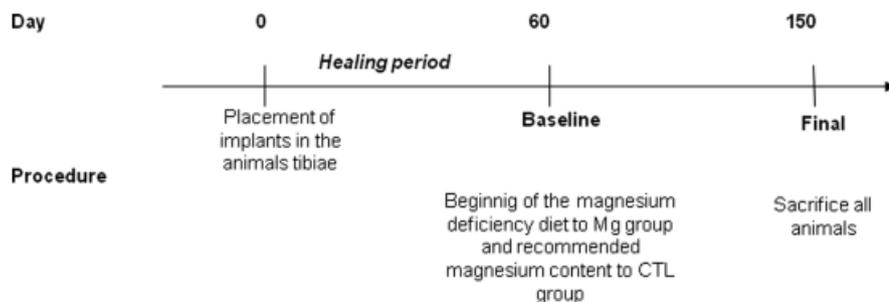


Fig. 1. Experimental design.



Fig. 2. (a) Regions of interest for analysis of radiographic bone density: 1 and 2 – upper cortical; 3 and 4 – cancellous bone; and 5 and 6 – lower cortical. (b and c) The radiographic aspect from control (CTL) and Mg groups.

### Removal torque test

Analysis of the removal torque of implants was immediately performed after the euthanization of the rats. The tibial implant was exposed, and the bone block was attached to a vise for stabilization. A 0.88 mm wrench was adapted to the internal connection of the implant and the torque was measured using a torque gauge (ATG24CN-S, Tohnichi MFG Co. Ltd., Tokyo, Japan) on a scale of 0.5 N cm with force ranging from 3 to 24 N cm. A reverse force was applied until complete rupture of the bone–implant interface, and the force needed to cause displacement of the implant in the bone tissue was recorded.

### Data analysis

Statistical analyses were performed using GraphPad Prism (GraphPad Prism 5.0, San Diego, CA, USA). Mann–Whitney test was conducted for comparisons between groups for all analyzed parameters. Significance level was set at 5%.

## Results

### Body weight

Analysis of the animal weight shows that there was a measurable gain for both groups during the experimental period. At study onset, a significant difference ( $P=0.0048$ ) between groups was shown. However, at baseline and the end of the experiment, no significant difference was found between groups (Fig. 3).

### Biochemical evaluations

Results showed a statistically significant decrease in magnesium serum levels for the Mg group when compared with control ( $P<0.0001$ ). However, for calcium serum levels, no difference was found (Fig. 4).

Serum levels of OCN showed no significant difference between groups; however, there was a tendency ( $P=0.0906$ ) for reduction in the OCN concentration when comparing the groups. Regarding PTH ( $P=0.0377$ ) and DPD ( $P=0.0303$ ) levels, there were statistically significant differ-

ences between groups disclosing higher values for the Mg group (Fig. 4).

### Bone densitometry

Bone densitometry showed significantly lower BMD values for lumbar vertebrae in the Mg group as compared with control group for all regions studied, L2 ( $P=0.0040$ ), L3 ( $P=0.0028$ ), L4 ( $P=0.0009$ ) and global ( $P=0.0010$ ) (Fig. 5).

### Analysis of radiographic bone density and cortical bone thickness

There was no difference between groups in radiographic bone density around implants (Fig. 6). On the other hand, upper ( $P<0.0001$ ) and lower ( $P=0.0436$ ) cortical thickness were shown to be significantly reduced for the Mg group compared with the control group (Fig. 7).

### Removal torque

Removal torque for the Mg group was significantly lower than the control group ( $P=0.0105$ ) (Fig. 8).

## Discussion

Systemic conditions, particularly those resulting in bone tissue changes, are viewed as important factors for implant treatment predictability (Marco et al. 2005). Within this respect, magnesium deficiency could be considered as a risk factor for osseointegrated implant, as it is recognized to affect bone metabolism (Creedon et al. 1999; Rude & Gruber 2004; Rude et al. 2004; Stendig-Lindberg et al. 2004).

In the present study, magnesium deficiency was induced for 3 months of dietary reduction of mineral content, demonstrated by its decreased serum levels. Calcium is a mineral antagonist to magnesium and in animals, there may be a tendency to increase the serum level in proportion to magnesium deficiency (Bussière et al. 2002; Meisel et al. 2005), which agrees with this study (Fig. 4).

Bone turnover is a physiologically complex process that involves not only interaction among

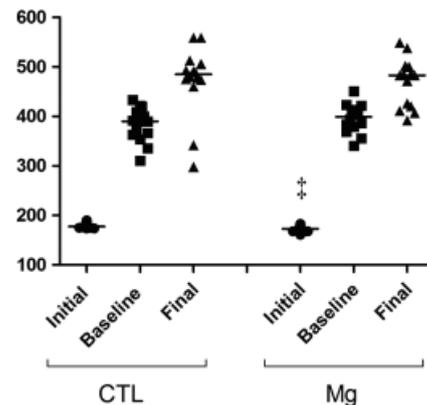


Fig. 3. Scattered distribution and median of body weight of the animals during experimental period ( $^{\dagger}P \leq 0.01$ ).

cells and bone matrix but also a variety of systemic and local regulation factors that coordinate cell proliferation and activity (Marco et al. 2005; Takayanagi 2005). Many studies report that magnesium deficiency has a particular influence on bone mass loss (Creedon et al. 1999; Rude et al. 2003, 2004, 2005, 2006; Rude & Gruber 2004). This effect was observed in diets with different magnesium contents ranging from lesser (50% of NR) to the most severe restrictions (0.04% of NR), showing higher bone mass loss when the deficiency is increased (Rude et al. 2003, 2004, 2005, 2006; Rude & Gruber 2004; Del Barrio et al. 2009). In the present study, bone densitometric analysis of lumbar vertebrae disclosed bone mass loss statistically significant for the Mg group as compared with the control (Fig. 5), agreeing with Rude et al. (2004).

Successful treatment with osseointegrated implants depends, among other factors, on the formation of a rigid anchorage to the bone, which provides biomechanical stability (Shibata et al. 2008). Different geometries and properties of cortical and cancellous bone may affect this stability by deformation of the bone crest around endosteal implants (Petrie & Williams 2007).

The effect of magnesium deficiency on bone tissue around implants with established osseointegration was evaluated in this study by radiographic bone density, measurement of

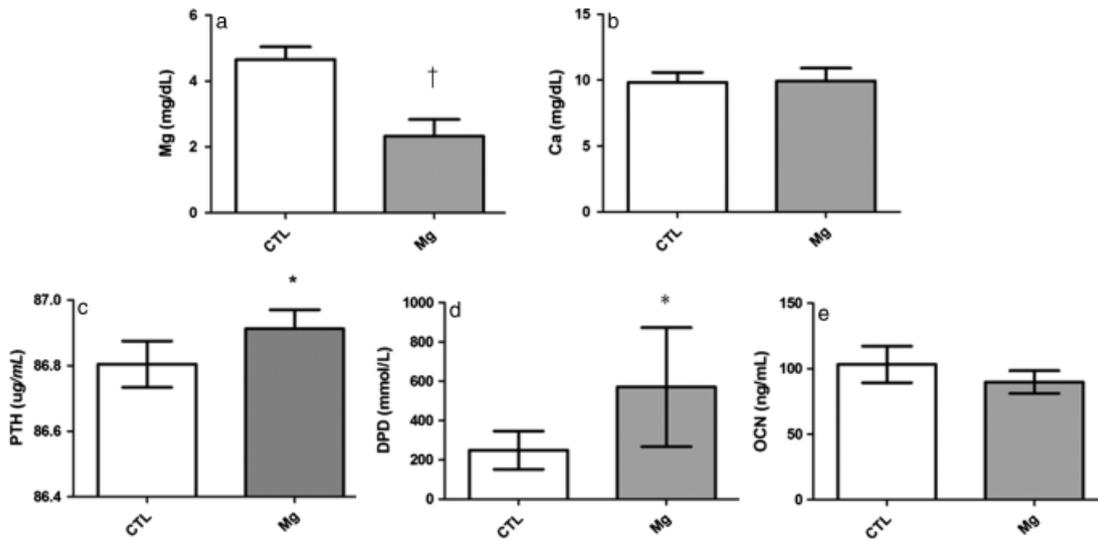


Fig. 4. (a) Serum levels of Mg ( $^{\dagger}P \leq 0.0001$ ), (b) Ca, (c) parathyroid hormone (PTH) ( $^*P \leq 0.05$ ) and (d) osteocalcin (OCN) and urine concentration of (e) deoxypyridinoline (DPD) ( $^*P \leq 0.05$ ) for groups control (CTL) and Mg.

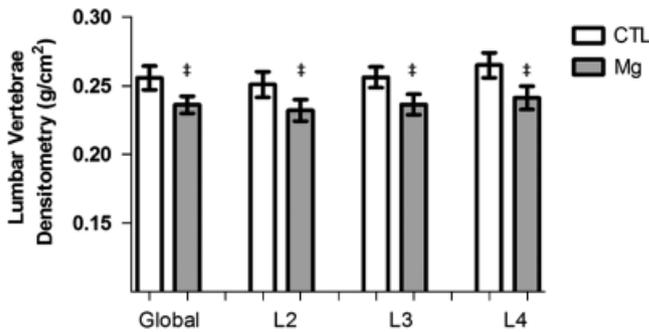


Fig. 5. Mean and 95% confidence interval of lumbar vertebrae bone densitometry in L2, L3, L4 and global ( $^{\#}P \leq 0.01$ ).

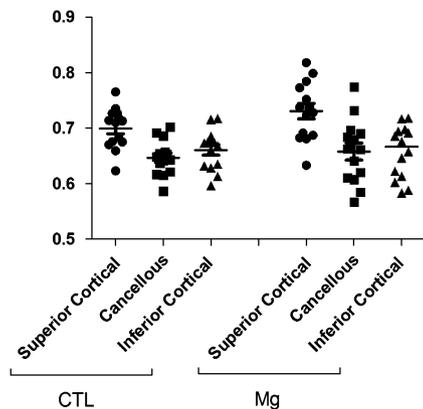


Fig. 6. Data distribution and median of radiographic bone density around implants in the regions of interest.

cortical bone thickness and removal torque. Results showed that magnesium deficiency had a negative effect on the peri-implant cortical bone significantly reducing tibial cortical thickness (Fig. 7).

Changes in cortical thickness greatly influences deformation of the peri-implant bone crest

when submitted to occlusal load. By finite element analysis, it was found that low-density cancellous bone and cortical bone thinning are more likely to undergo resorption when compared with bone tissue with thicker cortical and high-density cancellous bone, which could interfere in the treatment predictability (Petrie & Williams 2007).

However, an assessment of radiographic bone density around implants showed no significant difference between groups (Fig. 6). Although this methodology has been used previously to evaluate peri-implant bone tissue (Sakakura et al. 2006, 2008; Giro et al. 2008), in this study, the absence of difference between groups might be due to technique limitations when compared with DXA or the relatively short period of deficiency, resulting in changes not detected by this analysis.

Although no difference was found between groups for radiographic bone density, changes in bone morphology and size of hydroxyapatite crystals, affecting bone architecture, may have occurrence around implants in Mg group (Boskey

et al. 1992; Creedon et al. 1999; Rude et al. 2005, 2006). This uneven configuration of bone structure may cause structural weakness, creating microfractures of the trabeculae and changing biomechanical behavior. Such a structural change in hydroxyapatite crystals could not be detected by the related method, because the crystals' affected geometry may be masked under a bone structure with a radiographic density similar to that of the control group. However, such morphological changes together with a decreased cortical thickness may explain the difference in biomechanical behavior in view of the reduced implant removal torque for the Mg group.

Several mechanisms may induce bone mass decrease related to this deficiency, such as hormone regulation (PTH), stimulation of pro-inflammatory cytokines (IL-1 $\beta$  and TNF- $\alpha$ ) (Rude et al. 2003, 2004, 2005, 2006; Rude & Gruber 2004) and change in hydroxyapatite crystal formation (Boskey et al. 1992; Creedon et al. 1999; Rude et al. 2005, 2006). An increased release of PTH is related to an increase in calcium resorption by the kidneys, an increase in paracellular calcium and magnesium resorption through the stimulation of calcium channels. Thus, bone resorption, as a result of stimulation of new osteoclasts and an increased activity of mature ones, leads to imbalance of bone turnover (Holtrap et al. 1979; Goltzman 2008; Mosekilde 2008). A statistically significant difference between groups was found in this study by an assessment of this pathway, with higher values of serum PTH concentration for animals with deficiency (Fig. 4). An increased release of PTH was observed previously in mice with this deficiency, in short periods of reduced mineral intake

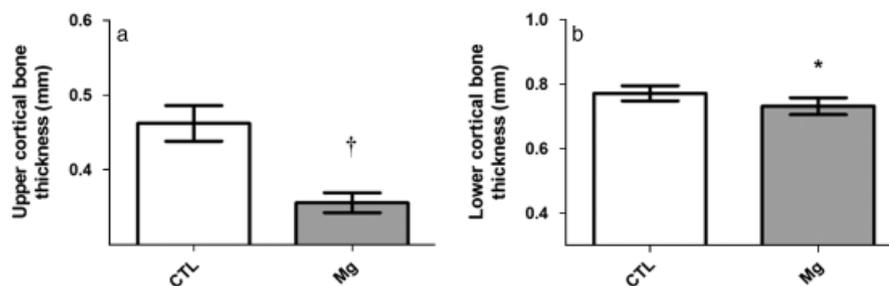


Fig. 7. Thickness of (a) upper ( $^{\dagger}P \leq 0.0001$ ) and (b) lower ( $^{*}P \leq 0.05$ ) cortical bone for groups control (CTL) and Mg.

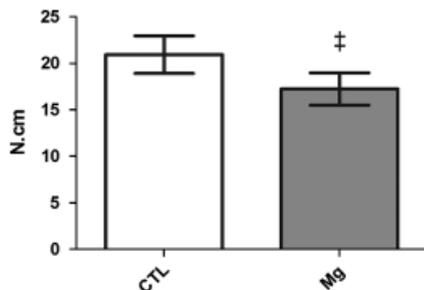


Fig. 8. Removal torque mean and 95% confidence interval of the implants for groups control (CTL) and Mg ( $^{\dagger}P \leq 0.01$ ).

[Rude et al. 2004, 2005, 2006]. Significantly higher values of urine DPD, an important bone resorption marker, were also verified in the group with magnesium deficiency. These data confirm the increased activity of bone resorption in this group. OCN expression, an important bone formation marker, is regulated by several calcitropic hormones and 1.25 dihydroxyvitamin D<sub>3</sub>, PTH, glucocorticoids and also by growth factors such as bone morphogenetic proteins, fibroblast growth factor 2 and TNF- $\alpha$  (Jiang et al. 2004). The decreased serum concentration

showed in the Mg group, although not statistically significant ( $P = 0.0906$ ) may be related to a decrease of its synthesis in magnesium deficiency (Carpenter et al. 1992). These findings express an imbalance on bone turnover in animals with magnesium deficiency, showing higher resorption activity without a concomitant increase in bone formation activity.

Within the limitations of this study, it was concluded that magnesium deficiency may lead to alterations on systemic bone metabolism, reduction of cortical bone thickness and lower removal torque of implants with established osseointegration. However, the related mechanisms to bone loss require further clarification.

**Acknowledgements:** This study was supported by the São Paulo State Research Support Foundation and The Coordination for Improvement of Higher Education Students. The authors gratefully acknowledge "Conexão Sistemas de Próteses Ltda" for supplying the implants used in this study.

## References

- Boskey, A.L., Rinnac, C.M., Bansal, M., Federman, M., Lian, J. & Boyan, B.D. (1992) Effect of short-term hypomagnesemia on the chemical and mechanical properties of rat bone. *Journal of Orthopaedic Research* **10**: 774–783.
- Bussière, F.I., Gueux, E., Rock, E., Manzur, A. & Rayssiguier, Y. (2002) Protective effect of calcium deficiency on the inflammatory response in magnesium-deficient rats. *European Journal of Nutrition* **41**: 197–202.
- Carpenter, T.O., Mackowiak, S.J., Troiano, N. & Gundberg, C.M. (1992) Osteocalcin and its messages: relationship to bone histology in magnesium-deprived rats. *The American Journal of Physiology* **263**: E107–E114.
- Clokic, C.M. & Warshawsky, H. (1995) Development of a rat tibia model for morphological studies of the interface between bone and titanium implant. *Compendium* **16**: 56, 58, 60.
- Creedon, A., Flynn, A. & Cashman, K. (1999) The effect of moderately and severely restricted dietary magnesium intakes on bone composition and bone metabolism in the rat. *The British Journal of Nutrition* **82**: 63–71.
- Del Barrio, R.A.L., Giro, G., Belluci, M.M., Pereira, R.M.R., Marcantonio, E. Jr & Orrico, S.R.P. (2009) Severe dietary magnesium deficiency decreased the systemic bone density and removal torque of osseointegrated implants. *International Journal of Oral & Maxillofacial Implants* (accepted).
- Evangeliopoulos, A.A., Vallianou, N.G., Panagiotakos, D.B., Georgiou, A., Zacharias, G.A., Alevra, A.N., Zalokosta, G.J., Vogiatzakis, E.D. & Avgerinos, P.C. (2008) An inverse relationship between cumulating components of the metabolic syndrome and serum magnesium levels. *Nutrition Research* **28**: 659–663.
- Ford, E.S. & Mokdad, A.H. (2003) Dietary magnesium intake in a national sample of US adults. *The Journal of Nutrition* **133**: 2879–2882.
- Giro, G., Gonçalves, D., Sakakura, C.E., Pereira, R.M.R., Marcantonio, E. Jr & Orrico, S.R.P. (2008) Influence of estrogen deficiency and its treatment with alendronate and estrogen on bone density around osseointegrated implants: radiographic study in female rats. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **105**: 162–167.
- Goltzman, D. (2008) Studies on the mechanisms of the skeletal anabolic action of endogenous and exogenous parathyroid hormone. *Archives of Biochemistry and Biophysics* **473**: 218–224.
- Grier, S.J., Turner, A.S. & Alvis, M.R. (1996) The use of dual-energy X ray absorptiometry in animals. *Investigative Radiology* **31**: 50–62.
- Holtrop, M.E., King, G.J., Cox, K.A. & Reit, B. (1979) Time-related changes in the ultrastructure of osteoclasts after injection of parathyroid hormone in young rats. *Calcified Tissue International* **27**: 129–135.
- Jiang, D., Franceschi, R.T., Boules, H. & Xiao, G. (2004) Parathyroid hormone induction of the osteocalcin gene. Requirement for an osteoblast-specific element 1 sequence in the promoter and involvement of multiple-signaling pathways. *The Journal of Biological Chemistry* **279**: 5329–5337.
- Johnson, S. (2001) The multifaced and widespread pathology of magnesium deficiency. *Medical Hypotheses* **56**: 163–170.
- Maguire, M.E. & Cowan, J.A. (2002) Magnesium chemistry and biochemistry. *Biometals* **15**: 203–210.
- Marco, F., Milena, F., Gianluca, G. & Vittoria, O. (2005) Peri-implant osteogenesis in health and osteoporosis. *Micron* **36**: 630–644.
- Marx, A. & Neutra, R.R. (1997) Magnesium in drinking water and ischemic heart disease. *Epidemiologic Reviews* **19**: 258–272.
- Meisel, P., Schwahn, C., Luedemann, J., John, U., Kroemer, H.K. & Kocher, T. (2005) Magnesium deficiency is associated with periodontal disease. *Journal of Dental Research* **84**: 937–941.
- Mosekilde, L. (2008) Primary hyperparathyroidism and the skeleton. *Clinical Endocrinology* **69**: 1–19.
- New, S.A., Robins, S.P., Campbell, M.K., Martin, J.C., Garton, M.J., Bolton-Smith, C., Grubb, D.A., Lee, S.J. & Reid, D.M. (1997) Dietary influences on bone mass and bone metabolism: further evidence of a positive link between fruit and vegetable consumption and bone health? *American Journal of Clinical Nutrition* **71**: 142–151.
- Petrie, C.S. & Williams, J.L. (2007) Probabilistic analysis of peri-implant strain predictions as influenced by uncertainties in bone properties and occlusal forces. *Clinical Oral Implants Research* **18**: 611–619.
- Reeves, P.G. (1997) Components of the AIN-93 diets as improvements in the AIN-76 diet. *Journal of Nutrition* **127**: 838S–841S.
- Reeves, P.G., Nielsen, F.H. & Fahey, G.C. Jr (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *Journal of Nutrition* **123**: 1939–1951.
- Rude, R.K. & Gruber, H.E. (2004) Magnesium deficiency and osteoporosis: animal and human observations. *The Journal of Nutrition Biochemistry* **15**: 710–716.
- Rude, R.K., Gruber, H.E., Norton, H.J., Wei, L.Y., Frausto, A. & Kilburn, J. (2005) Dietary magnesium reduction to 25% of nutrient requirement disrupts bone and mineral metabolism in the rat. *Bone* **37**: 211–219.

- Rude, R.K., Gruber, H.E., Norton, H.J., Wei, L.Y., Frausto, A. & Kilburn, J. (2006) Reduction of dietary magnesium by only 50% in the rat disrupts bone and mineral metabolism. *Osteoporosis International* **17**: 1022–1032.
- Rude, R.K., Gruber, H.E., Norton, H.J., Wei, L.Y., Frausto, A. & Mills, B.G. (2004) Bone loss induced by dietary magnesium reduction to 10% of the nutrient requirement in rats is associated with increased release of substance P and tumor necrosis factor- $\alpha$ . *Journal of Nutrition* **134**: 79–85.
- Rude, R.K., Gruber, H.E., Wei, L.Y., Frausto, A. & Mills, B.G. (2003) Magnesium deficiency: effect on bone and mineral metabolism in the mouse. *Calcified Tissue International* **72**: 32–41.
- Sabbagh, F., Lecerf, F., Hulin, A., Bac, P. & German-Fattal, M. (2008) Effect of hypomagnesemia on allogeneic activation in mice. *Transplant Immunology* **20**: 83–87.
- Sakakura, C.E., Giro, G., Gonçalves, D., Pereira, R.M.R., Orrico, S.R.P. & Marcantonio, E. Jr (2006) Radiographic assessment of bone density around integrated titanium implants after ovariectomy in rats. *Clinical Oral Implants Research* **17**: 134–138.
- Sakakura, C.E., Neto, R.S., Bellucci, M., Wenzel, A., Scaf, G. & Marcantonio, E. Jr (2008) Influence of homeopathic treatment with comfrey on bone density around titanium implants: a digital subtraction radiography study in rats. *Clinical Oral Implants Research* **19**: 624–628.
- Schimatschek, H.F. & Rempis, R. (2001) Prevalence of hypomagnesemia in an unselected German population of 16,000 individuals. *Magnesium Research* **14**: 283–290.
- Shibata, Y., He, L.H., Toda, Y., Kataoka, Y., Fujisawa, N., Miyazaki, T. & Swain, M.V. (2008) Micromechanical evaluation of mineralized multilayers. *Journal of Biomechanics* **41**: 3414–3418.
- Stendig-Lindberg, G., Koeller, W., Bauer, A. & Rob, P.M. (2004) Prolonged magnesium deficiency causes osteoporosis in the rat. *Journal of the American College of Nutrition* **23**: 704S–711S.
- Takayanagi, H. (2005) Inflammatory bone destruction and osteoimmunology. *Journal of Periodontal Research* **40**: 287–293.
- Touvier, M., Lioret, S., Vanrullen, I., Bocle, J.C., Boutron-Ruault, M.C., Berta, J.L. & Volatier, J.L. (2006) Vitamin and mineral inadequacy in the French population: estimation and application for the optimization of food fortification. *International Journal for Vitamin and Nutrition Research* **76**: 343–351.
- Tucker, K.L., Hannan, M.T., Chen, H., Cupples, L.A., Wilson, P.W. & Kiel, D.P. (1999) Potassium, magnesium, and fruit and vegetable intakes are associated with greater bone mineral density in elderly men and women. *The American Journal of Clinical Nutrition* **69**: 727–736.
- Wang, M.C., Moore, E.C., Crawford, P.B., Hudes, M., Sabry, Z.Y., Marcus, R. & Bachrach, L.K. (1999) Influence of pre-adolescent diet on quantitative ultrasound measurements of the calcaneus in young adult women. *Osteoporosis International* **9**: 532–535.